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Noonan syndrome and related disorders: dysregulated RAS-mitogen activated protein kinase signal transduction

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Noonan syndrome is a relatively common, genetically heterogeneous Mendelian trait with a pleiomorphic phenotype. Prior to the period covered in this review, missense mutations in *PTPN11* had been found to account for nearly 50% of Noonan syndrome cases. That gene encodes SHP-2, a protein tyrosine kinase that plays diverse roles in signal transduction including signaling via the RAS-mitogen activated protein kinase (MAPK) pathway. Noonan syndrome-associated *PTPN11* mutations are gain-of-function, with most disrupting SHP-2's activation–inactivation mechanism. Here, we review recent information that has elucidated further the types and effects of *PTPN11* defects in Noonan syndrome and compare them to the related, but specific, missense *PTPN11* mutations causing other diseases including LEOPARD syndrome and leukemias. These new data derive from biochemical and cell biological studies as well as animal modeling with fruit flies and chick embryos. The discovery of *KRAS* missense mutation as a minor cause of Noonan syndrome and the pathogenetic mechanisms of those mutants is discussed. Finally, the elucidation of gene defects underlying two phenotypically related disorders, Costello and cardio-facio-cutaneous syndromes is also reviewed. As these genes also encode proteins relevant for RAS-MAPK signal transduction, all of the syndromes discussed in this article now can be understood to constitute a class of disorders caused by dysregulated RAS-MAPK signaling.

INTRODUCTION

Noonan syndrome, named eponymously for the pediatric cardiologist who first described it, is an autosomal dominant disorder with features that include short stature, facial dysmorphia, pulmonary valve stenosis, pectus deformities and webbed or short neck (1). Less prevalent findings include cryptorchidism in affected boys, mental retardation, bleeding diatheses and hematopoietic abnormalities including certain leukemias. Like many autosomal dominant disorders, a significant percentage of cases arise from sporadic mutations. Noonan syndrome appears to be relatively prevalent among Mendelian conditions.

In 2001, Tartaglia *et al*. (2) discovered the first gene for Noonan syndrome, *PTPN11*. This gene encodes SHP-2, a nonmembranous protein tyrosine phosphatase with largely positive regulatory roles in signal transduction for many growth factors, cytokines and hormones. Of particular relevance to Noonan syndrome, SHP-2 has an established role in signaling through the well-known RAS-mitogen activated protein kinase (MAPK) pathway downstream of several receptor tyrosine kinases (Fig. 1). *PTPN11* mutations account for ~50% of Noonan syndrome cases.

In this review, we focus on information concerning Noonan syndrome that has emerged in the last 1–2 years. This includes efforts to understand the molecular spectrum of *PTPN11* mutations, particularly how it differs from those observed in other diseases, to discover new genes causing Noonan syndrome, and to elucidate the pathogenesis of Noonan syndrome because of mutant SHP-2 proteins. In addition, we review exciting discoveries of genes causing genetic disorders that phenotypically resemble Noonan syndrome. Taken together, this new knowledge has led to the recognition of a set of genetic syndromes whose etiology derives from dysregulated RAS-MAPK signaling.

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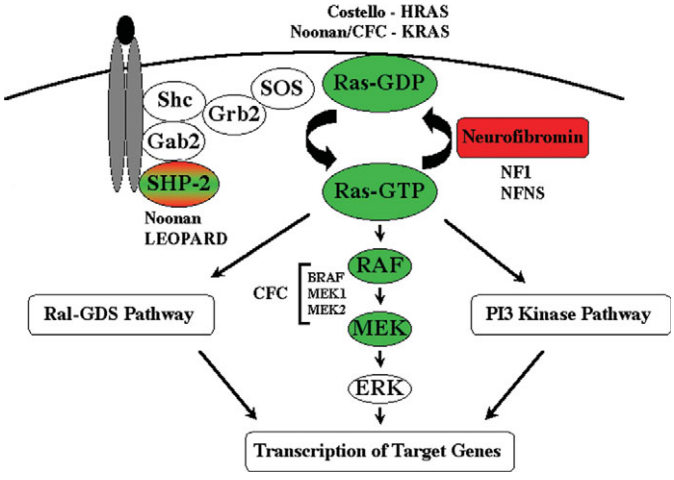

Figure 1. Schematic diagram showing the RAS-MAPK signal transduction pathway. The syndromes and their mutated proteins are as indicated: those with gain-of-function mutants are indicated in green and those with loss-of-function (neurofibromin) or dominant-negative effects (SHP-2) are shown in red. The double ovals in grey and black oval shown on the left represent a generic dimerized cell-surface receptor with its ligand. This figure was modified from one kindly provided by Kevin Shannon (University of California at San Francisco).

NOONAN SYNDROME

PTPN11 and Noonan syndrome: mutations and their effects

Available records based on more than 500 germline defects indicate that Noonan syndrome-causing *PTPN11* mutations are almost always missense changes and are not randomly distributed throughout the gene (3). We classified mutations into six major groups on the basis of their predicted effect on protein function (Table 1 and Fig. 2A). Most of the mutations affect residues involved in the N-SH2/PTP interdomain binding network that stabilizes SHP-2 in its catalytically inactive conformation or are in close spatial proximity to them. These mutations are predicted to up-regulate SHP-2 physiological activation by impairing the switch between the active and inactive conformation without altering SHP-2's catalytic capability. Recent biochemical data support this view (3,4). A number of mutations, however, affect residues contributing to the stability of the catalytically inactive conformation but also participating in catalysis or controlling substrate specificity. For a number of these defects it can be speculated that the individual substitution does not markedly perturb substrate affinity or catalysis, and that enzymatic activation by N-SH2 dissociation might prevail.

Somatic *PTPN11* mutations have been documented to contribute to leukemogenesis (5,6). The available molecular data indicate that specificity in the amino acid substitution is relevant to the functional deregulation of SHP-2 and disease pathogenesis (Table 1 and Fig. 2B). Indeed, comparison of the molecular spectra observed with the Noonan syndrome and leukemias indicate a clear-cut genotype/phenotype correlation, strongly supporting the idea that the germline transmitted *PTPN11* mutations have different effects on development and hematopoiesis than those acquired somatically. Consistent with this, the biochemical behavior of SHP-2 mutants associated with cancer tend to be more activating than observed with the Noonan syndrome-associated mutant proteins (3,4). Moreover, the leukemia-associated *PTPN11* mutations up-regulate RAS signaling and induce cell hypersensitivity to growth factors and cytokines more than the Noonan syndrome defects do (7,8). Overall, the available genetic, modeling, biochemical and functional data support a model in which distinct gain-of-function thresholds for SHP-2 activity are required to induce cell-, tissue- or developmental-specific phenotypes, each depending on the transduction network context involved in the phenotype.

New Noonan syndrome gene discovery–*KRAS*

Genetic linkage studies and *PTPN11* genotyping established that one or more additional Noonan syndrome genes exist. In the past year, two groups independently used a candidate gene approach to discover that *KRAS* mutations can cause Noonan syndrome (9,10). Four missense heterozygous mutations in the *KRAS* gene have been identified in seven individuals among 212 *PTPN11* mutation-negative subjects with Noonan syndrome (~3% of cases). Since *PTPN11* is mutated in about half of cases with the disorder, the prevalence of *KRAS* mutations in Noonan syndrome can be estimated at less than 2%. A variable phenotype was associated with germline *KRAS* mutations. One subject had JMML and craniosynostosis, and two exhibited clinical features overlapping Costello and cardio-facio-cutaneous (CFC) syndromes.

The *KRAS* gene produces two transcripts, encoding KRASA and KRASB, through alternative splicing (11). *KRASA* is expressed in a tissue- and temporally restricted fashion (12), whereas *KRASB* is expressed ubiquitously. With the other RAS family members, KRAS isoforms A and B share a structure that includes a conserved region, called the G domain (residues 1–165), which encompasses the motifs required for its signaling function, and a less conserved C-terminal tail, named the hypervariable region (11).

RAS proteins participate in multiple signal transduction pathways controlling cell proliferation, differentiation and survival and function as GDP/GTP-regulated molecular switches to control intracellular signal flow (13). RAS proteins cycle from a GDP-bound inactive state to a GTP-bound active state, the latter allowing signal flow by protein interaction with multiple downstream transducers. GDP/GTP cycling is controlled by GTPase activating proteins (GAPs) that accelerate the intrinsic GTPase activity and guanylyl exchanging factors (GEFs) that promote release of GDP. Two tracts in the G domain, called Switch I and Switch II, undergo major conformational changes on GTP/GDP exchange and mediate binding to GAPs and GEFs (13–15).

KRASA and KRASB have distinct C-terminal tails. In KRASA, this region contains a CAAX motif, and is palmitoylated at cysteine residues with resulting effects on membrane localization of the protein. In KRASB, the CAAX domain near the C-terminus is replaced with a polylysine stretch. This differential processing has profound functional effects, leading to alternative trafficking pathways to the plasma membrane and distinct membrane localization (11).

Table 1. Classification and relative distribution of germline and somatic *PTPN11* mutations

Mutation group[a]	Predicted effect on SHP-2 function	Germline origin ($N = 573$)		Somatic origin ($N = 256$)	
		N	%	*N*	%
I	A/I switching	243	42.4	217	84.8
II	A/I switching and catalysis	66	11.5	3	1.2
III	A/I switching and specificity	27	4.7	27	10.5
IV	A/I switching and/or catalysis	195	10.5	4	1.6
V	SH2 pY-binding	28	4.9	5	1.9
VI	SH2 orientation or mobility	12	2.1	–	–
Others	–	2	0.4	–	–

[a]Group I mutations affect PTP domain-interacting N-SH2 residues. Groups II, III and IV affect residues spotted in the PTP domain. Group V mutations affect residues located in the phosphotyrosyl-binding pocket of the N-SH2 or C-SH2 domain. Group VI mutations affect residues located within the linker connecting the N-SH2 and C-SH2 domain. A/I, active/inactive conformation; SH2, Scr homology 2 domain; pY, phosphotyrosyl-containing peptide.

The position of the four amino acid residues (Val^{14}, Thr^{58}, Val^{152} and Asp^{153}) that are altered by Noonan syndrome-associated *KRAS* mutations suggests two distinct pathogenetic mechanisms. Two mutant KRAS proteins, V14I and T58I, affecting the P-loop and Switch II in the G domain, respectively, have less GTPase activity, basally and after stimulation with a GAP protein, than wild-type KRAS but more than the oncogenic G12D (10). Thus, these mutants reduce RAS inactivation, resulting in increased RAS signaling. In this, they resemble the oncogenic KRAS mutants but with qualitatively less gain-of-function.

The other two Noonan syndrome-associated KRAS mutants, V152A and D153V, alter residues that reside far from the P-loop typically altered in cancer (16), suggesting a different effect on protein function. Our analysis of the structural consequences of the V152A and D153V mutants indicates that these substitutions destabilize the conformation of portions of the G domain that contribute extensively to the interaction of KRAS with the GTP/GDP guanine ring (9). Thus, we have predicted that the V152A and D153V mutants will have normal GTPase function but reduced binding to GTP and GDP, permitting GEF-independent activation. Biochemical study of these mutants is underway. Also, of note, the Val^{152} and Asp^{153} codons are encoded by exon 6, so the V152A and D153V mutations only alter KRASB, providing new evidence that this isoform plays a major role during development.

Animal models of Noonan syndrome

The elucidation of the pathogenesis of Noonan syndrome, particularly with respect to the developmental perturbations, will depend upon studies of animal models. Previously, a *bona fide* mouse model with the D61G mutation in *PTPN11* has been generated (17). Although mechanistic studies of Noonan syndrome mice are in progress, new information concerning gain-of-function Shp-2 and development has emerged through work with other species.

Fruit fly development

Oishi and *et al*. (18) modeled some Noonan syndrome and leukemia *PTPN11* mutations using transgenic fruit flies. The *Drosophila* homologue of *PTPN11* is *corkscrew* (*csw*) (19), an X-linked gene that is named for the shape of misshapen fly embryos that result from deficiency of this protein tyrosine phosphatase. Corkscrew acts downstream of several receptor tyrosine kinases including Torso (terminal structure development), Sevenless (photoreceptor R7 development in the *Drosophila* eye), Deranged (development of several structures including eyes, wings and legs), Breathless (trachea development) and Heartless (dorsal aortic development) (20–25).

Transgenic flies bearing Noonan syndrome- or leukemia-associated *csw* gain-of-function alleles were generated and the mutant proteins expressed using the UAS-Gal4 system (18). The mutant corkscrew proteins retained PTP activity as documented through rescue of the rough eye phenotype associated with a hypomorphic *csw* allele. Ubiquitous expression of the gain-of-function *csw* alleles did not perturb development downstream from all RTK pathways (e.g. development of the trachea and dorsal aorta were normal as was the expression domain of *tailless* as a readout for Torso signaling). Two strong *csw* alleles engendered embryonic or larval lethality, whereas expression of the weaker Noonan syndrome allele, N308D, resulted in ectopic wing vein formation. Activation of Ras was necessary but not sufficient for the expression of these phenotypes. As the ectopic wing vein phenotype closely resembled that observed with Egfr gain-of-function (26), epistatic studies with genes relevant for Egfr-Ras-Mapk signaling were performed (18). These showed that the N308D allele interacted genetically with nearly all genes in the pathway, documenting dependence on the activation of the receptor by its ligand for ectopic wing vein formation. Lastly, genetic interactions with the Notch, Decaptentaplegic (the orthologue of bone morphogenetic protein) and Jak/Stat signaling pathways were established.

Chick cardiac endocardial cushion development

Krenz *et al*. (27) modeled the effects of a Noonan syndrome-related *PTPN11* mutation through expression of Q79R mutant Shp-2 in valve primordia using the chick explant culture system and recombinant adenoviruses. Overexpression of the Q79R Shp-2 in atrioventricular (AV) and outflow tract endocardial cushion explants significantly augmented cell outgrowth due to increased cell proliferation, whereas wild-type Shp-2 did not have this effect. This outgrowth

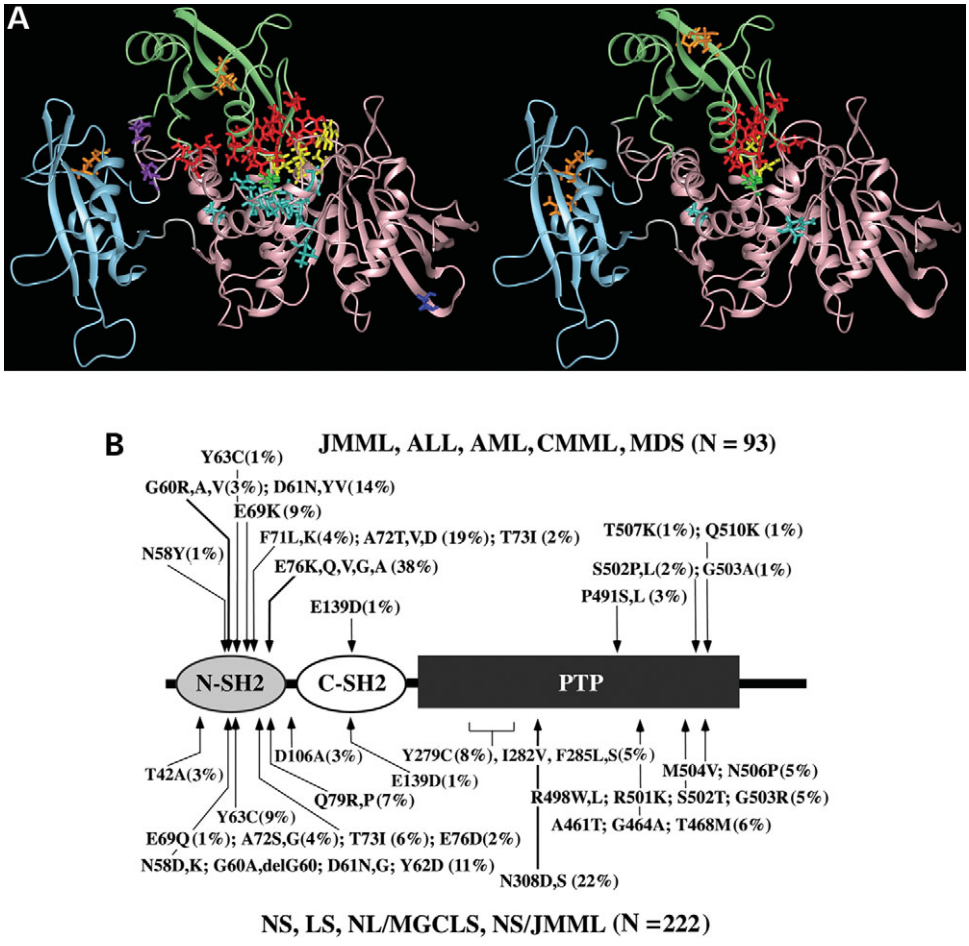

Figure 2. Comparison of *PTPN11* mutations causing Noonan syndrome and cancers. (**A**) Location of mutated residues in the three-dimensional structure of SHP-2 in its catalytically inactive conformation (green, N-SH2 domain; cyan, C-SH2 domain; pink, PTP domain). Residues altered in Noonan syndrome (left) or leukemias (right) are shown with their lateral chains colored according to the proposed classification (red, group I; yellow, group II; green, group III; cyan, group IV; orange, group V; violet, group VI; blue, unclassified) (3). Reprinted with permission from the *American Journal of Human Genetics*. (**B**) Distribution of SHP-2 mutations in human disease. Schematic representation of SHP-2 domain organization showing the distribution of mutations and their relative prevalence in hematologic malignancies (above), and Noonan syndrome and related developmental disorders (below). JMML: juvenile myelomonocytic leukemia; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CMML: chronic myelomonocytic leukemia; MDS: myelodysplastic syndromes. Reprinted with permission from *Annual Reviews in Genomics and Human Genetics*.

effect of Q79R could be blocked by inhibiting signaling through the Mapk Erk1/2, either pharmacologically with an Erk1/2 kinase (Mek-1) inhibitor or through co-expression of a dominant-negative form of Mek-1. Expression of a constitutively active form of Mek-1 phenocopied the effects of Q79R Shp-2. Pharmacological inhibition of an alternative Mapk pathway that uses p38 had no effect on the Q79R-induced outgrowth. Lastly, application of cyclosporine A, which blocks calcineurin signaling, also failed to alter the endocardial cushion outgrowth. Taken together, these experiments document that Noonan syndrome-associated *PTPN11* mutations induce increased cell proliferation during endocardial cushion development because of increased signaling via the Ras-Mapk pathway using Erk1/2. While calcineurin/NFATc signaling has an established role in epithelial–mesenchymal transdifferentiation during cardiac valvulogenesis (28), there does not appear to be crosstalk with that pathway and Erk1/2 signaling driven by gain-of-function Shp-2 during the proliferative phase of valve development.

NOONAN SYNDROME-RELATED DISORDERS

Clinical geneticists have recognized several disorders that resembled Noonan syndrome phenotypically. The discovery of *PTPN11* as a Noonan syndrome gene empowered studies addressing the question of whether those disorders were also related genetically. Although LEOPARD syndrome did turn out to be allelic (29,30), Costello and CFC syndromes did not (31–34). Within the past year, however, several studies have shown that these latter two disorders are related mechanistically because, like Noonan syndrome, they result from dysregulated RAS-MAPK signaling. Of note, *PTPN11* mutations causing LEOPARD syndrome appear to dysregulate RAS-MAPK signaling in a different manner.

LEOPARD syndrome

LEOPARD syndrome is an autosomal dominant disorder that overlaps phenotypically with Noonan syndrome. The

acronymic name refers to the major features: *L*entigines, *E*CG conduction abnormalities, *O*cular hypertelorism, *P*ulmonic stenosis, *A*bnormal genitalia, *R*etardation of growth and sensorineural *D*eafness (35). Two groups independently identified *PTPN11* missense mutations in patients with LS (29,30). Y279C and T468M represent the most common defects, although additional mutations (A461T, G464A, R498W, Q506P and Q510E) have been documented (29,36–40). A substantial decrease in phosphatase activity has been documented for three recurrent mutations (3,41,42). Moreover, results from expression studies of mutants in cell culture were consistent with a dominant negative mechanism. Additional work is needed to explain how the diminished catalytic activity of these SHP-2 mutants results in a condition phenotypically similar to Noonan syndrome.

Costello syndrome

Costello syndrome is a generally sporadic disorder overlapping in phenotype with Noonan syndrome but characterized by, generally, more severe mental retardation, coarse facial features, papillomata in the peri-oral and peri-nasal areas and a proclivity to certain solid organ cancers. Using a candidate gene approach, Aoki *et al*. (43) discovered that missense *HRAS* mutations cause Costello syndrome. These germline mutations altered residues Gly^{12} and Gly^{13}in HRAS's P-loop and had been identified previously as somatic defects in various tumors. As with the KRAS P-loop mutants discussed earlier, these HRAS mutants cause gain-of-function due to impairment of the GTPase activity that is necessary to inactivate RAS-GTP (44). Studies with skin fibroblasts from four individuals with Costello syndrome revealed increased growth factor-induced cell proliferation (43).

The molecular epidemiology of *HRAS* mutations in Costello syndrome has been explored (45–47). *HRAS* mutations were noted in greater than 85% of individuals diagnosed with Costello syndrome. Among the mutations, which were invariably sporadic, G12S was strongly predominant and accounted for ~85% of the lesions. Although missense substitutions more frequently alter Gly^{12} than Gly^{13} in cancer, G12S constitutes 5% and G12V predominates among those somatic Gly^{12} mutations. Combined with the biochemical results, these genetic epidemiological data suggest that less gain-of-function of HRAS is needed for the developmental perturbations than for the oncogenesis. Finally, two missense *HRAS* mutation, K117R and A146T, have been observed affecting a different portion of the protein (47 and our unpublished data). Of note, both substitutions altered the same portion of RAS as did the V152G and D153V *KRAS* defects, a domain that is relevant for binding the guanine ring of GDP and GTP (9).

CFC syndrome

CFC syndrome is a sporadic disorder that also resembles Noonan syndrome. Distinguishing features of CFC include a distinctive facial appearance with high forehead, bitemporal narrowing and supraorbital hypoplasia and ectodermal abnormalities including keratosis, ichthyosis, as well as sparse and easily fractured hair. Using a candidate gene approach based on the information on RAS-MAPK alterations in Noonan and Costello syndromes, two groups recently identified genes that are mutated in CFC (48,49). *BRAF*, which encodes a MAPK kinase kinase (Fig. 1), was mutated in slightly more than half of the patients who were genotyped. All of the molecular defects were missense changes and clustered in the cysteine-rich and protein kinase domains. Functional assessment of several missense BRAF mutants revealed that some increased its kinase activity, whereas others reduced it, similar to what has been observed among cancer-related BRAF mutants (49). Expression of activating BRAF mutants in cell culture resulted in activation of the MAPK ERK1/2 and activation of the transcription factor ELK, which resides downstream, whereas expression of the loss-of-function mutants had opposite effects (48,49). One group identified a few mutations in *MEK1* and *MEK2*, which encode MAPK kinases (Fig. 1) (49). When expressed, the mutants consistently displayed a gain-of-function. Finally, the other group reported three *KRAS* mutations among their cohort of 43 CFC subjects (48). Two individuals had the D153V substitution, which has been reported in severe NS; the other mutation, G60R, resides close to the T58I change in Switch II associated with NS (9,10). Of note, the three patients with CFC and *KRAS* mutations did not have typical skin involvement (more than two-thirds of those with *BRAF* defects did). This suggests that the phenotype associated with *KRAS* mutations may be distinct, residing at the interface between severe NS and mild CFC.

CONCLUDING REMARKS

In recent years, we have witnessed the elaboration of genetic causes of Noonan syndrome and several related syndromes, which can now be classed at disorders of dysregulated RAS-MAPK signaling. Further studies are needed to identify other genes, probably functionally related, that also cause Noonan syndrome and to elaborate disease pathogenesis. These steps are preludes to developing improved diagnostics and therapeutics for these genetic disorders.

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Conflict of Interest statement. Both authors receive royalties from the licensing of the invention of PTPNII mutations in Noonan syndrome from several companies peforming clinical mutation testing.

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